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(54) Title: POLYPHENOL FRACTIONS OF TEA, THE USE THEREOF AND FORMULATIONS CONTAINING THEM

(57) Abstract

The present invention relates to the preparation of novel polyphenol fractions of *Camellia sinensis* (tea), the use thereof and formulations containing them. The invention relates specifically to the preparation of extracts deprived of caffeine but containing the polyphenols deriving from epigallocatechin in a natural ratio. These extracts can be used alone or combined with lipophilic antioxidants, sulfurated amino acids and oligoelements of biochemical and nutritional importance. The use of these novel extracts, alone or in combination with other active principles, is of interest to the food, pharmaceutical and cosmetic fields.

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POLYPHENOL FRACTIONS OF TEA, THE USE THEREOF AND FORMULATIONS CONTAINING THEM

The present invention relates to novel polyphenol fractions of Camellia sinensis (tea), the process for the preparation thereof and the use thereof, as well as formulations containing them.

5 The object of the invention relates particularly to extracts deprived of caffeine, but containing mainly the polyphenols deriving from epigallocatechin in their natural ratio.

10 Caffeine is known to have undesired effects on the cardiovascular system as well as a mutagenic effect, and it is usually removed by extraction with carbon dioxide in hypercritical phase or chlorinated solvents; however, said procedures are not aimed at keeping a standardized content in all those polyphenol components responsible
15 for beneficial biological effects.

20 These substances, characteristic of the tea plant, have recently been the object of a great interest due to their strong antioxidant effect, as described in literature (1,2). Such an effect is apparently related to some extent to the capability of some of these polyphenols (for example epigallocatechin-3-O-gallate) of inhibiting the formation of neoplasias artificially induced in the laboratory animals (3,4).

25 Moreover, some recent epidemiological studies evidenced how the consumption of large amounts of green tea is associated with a lower incidence of degenerative chronic diseases, for example some forms of cancer and atherosclerosis (5-8).

Up to now, the mechanisms of action of the substances contained in green tea derivatives have not yet been completely elucidated, apart from the general antioxidant effect described above. The Applicant, during extensive studies, found that the polyphenol component of green tea is capable of exerting a differentiated cytotoxic effect depending on the type of the studied cell and that such a differentiation is particularly related to the selective targeting which some of these polyphenol molecules have towards sub-cell sites such as mitochondria.

Therefore, the importance of an extraction method allowing to obtain extracts of Camellia sinensis characterized by a standardized content in polyphenol substances and by a low caffeine content, is evident.

Such an aim has been attained by means of a process which comprises:

- a) extraction of the vegetable biomass with water-alcohols or water-ketones mixtures;
- 20 b) total or partial evaporation of the extraction solvent;
- c) suspension of the residue in an aqueous mixture of 30-60% by volume methanol;
- d) extraction of the hydromethanol suspension with chlorinated solvents;
- 25 e) filtration of the insoluble residues and concentration of the hydromethanol phase;
- f) optional acidification with organic acids;
- g) extraction of the concentrated hydromethanol phase and optional acidification with solvents selected from aliphatic esters, alcohols or ketones.
- 30

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provided that they are immiscible with the hydromethanol mixture;

- h) optional addition of an aromatic or aliphatic hydrocarbon, followed by washing with diluted aqueous mineral acids;
- i) concentration of the water-immiscible organic phase and dilution with chlorinated solvents;
- k) in the absence of steps f) and h), treatment with macromolecular cation strongly acidic exchange resins in dry aliphatic ketones or alcohols, preferably alcohols.

According to a first embodiment of the process of the invention, the vegetable biomass is extracted with aliphatic alcohols and ketones diluted with water or alone, preferably with aqueous acetone mixtures ranging from 40 to 90% by volume, (particularly with 70% acetone). The resulting extracts are concentrated, depending on the solvent used, either to small volume (from about 1/5 to about 1/15 starting volume) or to elimination of the organic solvent.

In the first case, the aqueous concentrate is diluted with methanol to 50% (v/v), whereas in the second case the residue is suspended in an aqueous methanol mixture ranging from 30 to 60%, preferably 50%. The hydromethanol mixture is counter-extracted with chlorinated solvents, preferably methylene chloride, until elimination of undesired substances such as chlorophyll, terpenes and caffeine not complexed with the oligomeric polyphenols present in the vegetable biomass. The chlorinated organic phases are discarded, whereas the hydromethanol phase is concentrated until

methanol is removed, filtering any insolubles consisting of tannin complexes with caffeine. The aqueous solution is extracted with immiscible solvents such as aliphatic ketones and alcohols or aliphatic esters. Examples of said solvents comprise methyl ethyl ketone in the presence of salts, such as ammonium chloride or sulfate (which can cleave the complexes with caffeine, due to the acidic and saline nature, keeping it in water and at the same time making a selective extraction of the polyphenols possible), butanol in its isomeric forms, ethyl formate or acetate. These solvents, preferably ethyl acetate, allow for a selective recovery of the phenol substances after concentration and dilution with chlorinated solvents, in particular methylene chloride. The resulting extracts still contain an amount of residual caffeine complexed with polyphenols higher than 1%, which can be removed by absorption on sulfonic resins in an anhydrous medium, using solvents such as methanol, ethanol or acetone, thereby obtaining an extract containing no more than 0.2% of caffeine.

In a further embodiment, the process of the invention comprises the extraction of the vegetable biomass with 40-50% (v/v) aqueous methanol or acetone, concentrating the eluate until elimination of the organic solvent, preferably to about the same weight as that of the extracted vegetable material. This concentrate, after filtering off the insolubles, is counter-extracted with chlorinated solvents, preferably methylene chloride, to remove the free caffeine and the terpene-like inert substances. The aqueous solution is acidified with organic acids, preferably citric acid in

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the presence of ammonium citrate, and counter-extracted with aliphatic esters, preferably ethyl acetate. The organic phase is added with aromatic or aliphatic hydrocarbons in a percentage from 2 to 20% by volume, preferably toluene in a 5% amount by volume based on the total volume of the organic phase, then washed with diluted mineral acids, preferably 1% sulfuric acid, until caffeine is removed.

The organic solution, after washing to neutrality, is concentrated to small volume and poured into a methylene chloride amount sufficient to recover the polyphenols.

The leaves of Camellia sinensis, preferably finely ground are used as vegetable biomass. The extractions are carried out at room temperature (approximately 18-25°C). The drug/solvent ratio is not critical, but it is generally comprised between 1:1 and 1:5 w/v for each single extraction.

The products obtained according to this process have on the average a content in the most important catechin derivatives of: 50-65% epigallocatechin-3-O-gallate, 13-20% epicatechin-3-O-gallate, 2-4% epicatechin and 1.5-3% epigallocatechin.

The polyphenol fractions obtained according to one of the reported methods are characterized by an antioxidant power comparable to or higher than that observed for known antioxidants, as it is evident from the following Table.

In this "in vitro" test, the anti-lipoperoxidant activity of the products was tested using an experimental model comprising sonication of phosphatidyl

choline liposomes in a test tube, and measurement of the products from their oxidative degradation by means of known techniques (9).

The incubation of liposomes in the presence of antioxidants decreases with a dose-related relationship the formation of conjugated dienes, which are a preliminary step of the oxidative degradation of the phospholipids.

TABLE

Antioxidant activity of the polyphenol fraction of green tea on sonicated phosphatidyl choline liposomes (propagation phase). Comparison with epigallocatechin-3-O-gallate, vitamin E and vitamin C

15

CI50 (μ M)

20

Green tea polyphenol fraction*

0.52

Epigallocatechin-3-O-gallate

0.50

Vitamin E

1.25

Vitamin C

inactive

* assuming as molecular weight that of epigallocatechin-3-O-gallate

25

Data are expressed as product concentrations (μ M) required to decrease by 50% (CI50) the formation of conjugated dienes following sonication of phosphatidyl choline liposomes.

The antioxidant activity of the polyphenols is believed to be also important as far as the antimutagenic effect is concerned, which has recently

been described for some polyphenols such as those extracted from grape-seeds (10).

Surprisingly, the polyphenols obtained from green tea (though having a high antioxidant activity), at the same concentrations as those antimutagenic for the products extracted from grape-seeds were antimutagenic, turned out to be devoid of said activity; on the other hand, they are characterized by a differential cytotoxicity (higher in cell lines in which a mitochondrial mutation is lethal) considered of great interest.

Said cytotoxicity is different depending on the cell lines used; in tumoral ovary cells, for example, the product of the present invention exerts a cytotoxic effect at concentrations of about 50 μM , whereas said effect can be measured at much higher concentrations (1000 μM) when evaluated on normal ovary cells.

In the present state of the art, different hypothesis have been formulated to elucidate the mechanisms on the basis of the spontaneous mutation, and among them ground has gained the assumption of some metabolic products of the cell cycle, particularly free radicals, being responsible for the modulation of the mitochondrial heredity through peroxidation of the membrane system on which the mitochondrial DNA replication depends (11,12).

With these premises, two classes of compounds are considered very interesting, which even though they use at least partly analogous basic mechanisms (antioxidant activity at the mitochondrion level), act on two different fronts: the antimutagenic (as it is the case

of grape-seed derivatives) and the differentiate
cytotoxic one (as it is the case of the products of the
present invention). Both classes of products can
therefore be used in the prophylactic treatment of
5 degenerative diseases such as neoplasias, cardiovascular
diseases such as atherosclerosis and arthritic and
arthrosis forms of various origin.

Moreover, the products of the present invention can
be used in therapy, alone or combined with other
10 substances so as to exert a synergistic effect. In
oncology, for example, the products can be combined with
the usual chemotherapy with the double advantage of both
diminishing the oxidative damage which generally is
involved in some treatments (for example with some
15 anthracyclines or platinum complexes, such as cis-
platinum) and destroying a part of the tumor cells,
through a differential cytotoxic mechanism.

In the prevention of the neoplastic event, the
polyphenol fraction taken with the diet is an effective
20 means to destroy mutated cells in the pre-cancer
condition. In other conditions, such as atherosclerosis,
the products of the invention can advantageously be
combined with other antioxidants such as carotenoids,
particularly lycopene and zeaxanthin in order to
25 preserve and maintain the integrity of the physiological
antioxidant pool.

In articular degenerative conditions, the products
of the present invention can be combined with sulfurated
amino acids such as methionin, cysteine or proline and
30 hydroxyproline and optionally administered in admixture
with glucosamine and jaluronic acid. The role played by

the polyphenol fraction in said combination is both antioxidative and antidegenerative on chondroblasts, due to the stimulation of synthesis of collagen and proteoglycans, in the presence of synergizing agents.

5 The active dosages of these extracts range from 10 to 1000 mg/die, one to four times a day, preferably 50 to 300 mg/day one to twice a day. The oral DL_{50} is above 2000 mg/kg in the rat and mouse.

10 The products can be incorporated in the conventional pharmaceutical forms, such as soft- or hard- gelatin capsules, tablets, sachets, syrups, suppositories and vials. In case of combination with other active principles, the compositions of the invention will optionally be suitable for the sequential
15 or separate administration of the single active principles.

The following examples further illustrate the invention.

20 Example I - Preparation of the polyphenol-standardized content decaffeinated extract of green tea.

1 kg of non-fermented, finely ground leaves of Camellia sinensis are extracted 4 times with 3 l each of an acetone/water 7:3 (v/v) mixture. The combined
25 extracts are concentrated to 1 kg under vacuum at a temperature not higher than 45°C. During the concentration, an abundant gummy mass forms, consisting of chlorophyll and other undesired lipophilic substances which anyhow contain polyphenols. The concentrate is
30 diluted with 1 l of methanol and 0.5 l of methylene chloride. The gummy mass is dissolved in the medium and, after 30' stirring, the phases are separated; the

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extraction with methylene chloride is repeated 3 more times and then the chloromethylene phase is discarded. The hydromethanol phase is concentrated under vacuum until elimination of methanol and the concentrate is extracted 3 times with 0.5 l of ethyl acetate. The aqueous phase is discarded whereas the organic one is dried over Na_2SO_4 and concentrated to small volume (0.22 l). The concentrate is poured into 1.2 l of methylene chloride under strong stirring to obtain an abundant brownish precipitate which, after drying under vacuum, weighs about 0.12 kg. This residue contains 1.6% of alkaloids and is subjected to a final purification dissolving it in 1.5 l of methanol, under nitrogen atmosphere. The methanol solution is treated with 250 ml of sulfonic resin Amberlyst[®] until disappearance of caffeine, which is checked by thin layer chromatography. The methanol solution is concentrated to dryness under vacuum at a temperature not above 50°C. According to this procedure, 0.11 kg of a beige product is obtained, having the following composition:

Epigallocatechin-3-O-gallate	58.0%
Epicatechin-3-O-gallate	16.0%
Epicatechin	3.0%
Epigallocatechin	2.5%

Example II - Preparation of the polyphenol-standardized content decaffeinated extract of green tea.

1 kg of non-fermented, finely ground leaves of Camellia sinensis are extracted 4 times with 3 l each of an acetone/water 4:6 mixture. The combined extracts are concentrated to 1 kg under vacuum at a temperature not above 45°C. During the concentration, some precipitate

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forms which is separated and discarded. The concentrate is extracted three times with 0.5 l of methylene chloride and the chloromethylene phase is then discarded. The aqueous phase is acidified to pH 1.5 with citric acid in the presence of ammonium citrate and extracted 3 times with 0.5 ml of ethyl acetate. The aqueous phase is then discarded whereas the organic one is diluted with 75 ml of toluene and counter-extracted 3 times with 150 ml each of 1% H_2SO_4 . After washing with water to neutrality and drying over Na_2SO_4 , the organic phase is concentrated to 300 ml and the concentrate is then poured into 1.5 ml of methylene chloride. After drying under vacuum overnight, 0.1 kg of a beige powder is obtained, having the characteristics of the product of example I.

Example III - Preparation of the polyphenol-standardized content decaffeinated extract of green tea.

1 kg of non-fermented, finely ground leaves of Camellia sinensis are extracted 4 times with 3 l each of a methanol/water 1:1 mixture. The combined extracts are concentrated to 1 kg under vacuum at a temperature not above 45°C. During the concentration, some precipitate forms which is separated and discarded. The concentrate is extracted three times with 0.5 l of methylene chloride and the chloromethylene phase is then discarded. The aqueous phase is acidified to pH 1.5 with citric acid in the presence of ammonium citrate and extracted 3 times with 0.5 l of ethyl acetate. The aqueous phase is then discarded whereas the organic one is diluted with 75 ml of toluene and counter-extracted 3 times with 150 ml each of 1% H_2SO_4 . After washing with

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water to neutrality and drying over Na_2SO_4 , the organic phase is concentrated to 300 ml and the concentrate is then poured into 1.5 ml of methylene chloride. After drying under vacuum at 50°C overnight, 0.1 kg of a beige powder is obtained, having the characteristics of the product of example I.

Example IV - Gelatin capsules containing the polyphenol fraction of green tea.

50 mg of polyphenol fraction of green tea are mixed with 88 mg of microcrystalline cellulose (Avicel PH 102), 3 mg of colloidal silica (Aerosil 200), 8 mg of cross-linked sodium carboxymethyl cellulose (Ac-Di-Sol) and 1 mg of magnesium stearate. The resulting mixture is encapsulated in hard-gelatin capsules (Size 3).

Example V - Gelatin capsules containing the polyphenol fraction of green tea.

150 mg of polyphenol fraction of green tea are mixed with 264 mg of microcrystalline cellulose (Avicel PH 102), 9 mg of colloidal silica (Aerosil 200), 24 mg of cross-linked sodium carboxymethyl cellulose and 3 mg of magnesium stearate. The resulting mixture is encapsulated in hard-gelatin capsules (Size 0).

Example VI - Coated tablets containing the polyphenol fraction of green tea.

50 mg of polyphenol fraction of green tea are mixed with 50 mg of microcrystalline cellulose (Avicel PH 101), 33 mg of dibasic calcium phosphate (Emcompress A), 3 mg of colloidal silica (Aerosil 200), 12 mg of cross-linked sodium carboxymethyl cellulose (Ac-Di-Sol) and 2 mg of magnesium stearate. The powders are mixed for 10 minutes, then tableted dosing at 150 mg/tablet. The

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resulting tablets (diameter: 7 mm) are coated with a film layer containing 4.5 mg of hydroxypropylmethyl cellulose (Methocel E5), 0.9 mg of Polyethylene glycol 6000 (Carbowax 6000), 1.2 mg of titanium dioxide and 0.9 mg of talc.

Example VII - Coated tablets containing the polyphenol fraction of green tea.

150 mg of polyphenol fraction of green tea are mixed with 150 mg of microcrystalline cellulose (Avicel PH 101), 99 mg of dibasic calcium phosphate (Emcompress A), 9 mg of colloidal silica (Aerosil 200), 36 mg of cross-linked sodium carboxymethyl cellulose (Ac-Di-Sol) and 6 mg of magnesium stearate. The powders are mixed for 10 minutes, then tableted dosing at 450 mg/tablet.

The resulting tablets (diameter: 11 mm) are coated with a film layer containing 13.5 mg of hydroxypropylmethyl cellulose (Methocel E5), 2.7 mg of Polyethylene glycol 6000 (Carbowax 6000), 3.6 mg of titanium dioxide and 2.7 mg of talc.

Example VIII - Vials containing the polyphenol fraction of green tea.

10 mg of polyphenol fraction of green tea are mixed with 200 mg of glucose and dissolved in water for injectable preparations q.s. to 5 ml. This solution (pH 4.5) can then be used for the preparation of injectable vials to be prepared with conventional techniques.

References

- 1 - Zhao B. et al., Cell Biophys. 14, 175, 1989
- 2 - Huang M.T. et al., Carcinogenesis 13, 947, 1992
- 3 - Conney A.H. et al., Prev. Med. 21, 361, 1992
- 4 - Mukhtar H. et al., J. Invest. Dermatol. 102, 3, 1994

- 5 - Kono S. et al., Jpn. J. Cancer Res. 79, 1067, 1988
- 6 - Oguni I. et al., Agric. Biol. Chem. 52, 1879, 1988
- 7 - Kato I. et al., Jpn. J. Cancer Res. 81, 1101, 1990
- 8 - Kono S. et al., Prev. Med. 21, 526, 1992
- 5 9 - Maffei-Facino R. et al., Arzneim.-Forsch./Drug Res.
44, 592, 1994
- 10 - Liviero L. et al., Fitoterapia LXV, 203, 1994
- 11 - Dujon B., in "Mitochondrial genetics and
functions", Straton J.N. et al. (Eds.), Cold Spring
10 Harbor Laboratory, Cold Spring Harbor, N.Y., 1981
- 12 - De Flora S. et al., Mutation Res. 214, 153, 1989.

CLAIMS

1. A process for the extraction of polyphenol fractions of Camellia sinensis containing no more than 0.2% by weight of caffeine, which process comprises:

- a) extraction of the vegetable biomass with water-alcohols or water-ketones mixtures;
- b) total or partial evaporation of the extraction solvent;
- 10 c) suspension of the residue in an aqueous mixture of 30-60% by volume methanol;
- d) extraction of the hydromethanol suspension with chlorinated solvents;
- 15 e) filtration of the insoluble residues and concentration of the hydromethanol phase;
- f) optional acidification with organic acids;
- g) extraction of the concentrated hydromethanol phase and optional acidification with solvents selected from aliphatic esters, alcohols or ketones, provided that they are immiscible with the hydromethanol mixture;
- 20 h) optional addition of an aromatic or aliphatic hydrocarbon, followed by washing with diluted aqueous mineral acids;
- 25 i) concentration of the water-immiscible organic phase and dilution with chlorinated solvents;
- k) in the absence of steps f) and h), treatment with sulfonic resins in dry aliphatic ketones or alcohols.

30 2. A process according to claim 1, wherein 40 - 90% by volume aqueous acetone is used in step a).

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3. A process according to claim 2, wherein 70% by volume aqueous acetone is used.
4. A process according to any one of claims 1 to 3, wherein 50% by volume aqueous methanol is used in step c).
5. A process according to any one of claims 1 to 4, wherein methylene chloride is used in step d).
6. A process according to any one of claims 1 to 5, wherein citric acid in the presence of ammonium citrate is used in step f).
7. A process according to any one of claims 1 to 6, wherein the solvents of step g) are selected from ethyl acetate, ethyl formate, methyl ethyl ketone, 1-butanol, tert-butanol, 2-butanol.
8. A process according to claim 7, wherein the solvent is ethyl acetate.
9. A process according to any one of the above claims, wherein toluene in an amount of 5% by volume based on the total volume of the organic phase and 1% by volume aqueous sulfuric acid are used in step h).
10. A process according to any one of the above claims, wherein methylene chloride is used in step i).
11. A process according to any one of claims 1 to 10, comprising the steps a) to e), g), i) and k).
12. A process according to any one of claims 1 to 10, comprising the steps a) to i).
13. Polyphenol fractions of Camellia sinensis containing no more than 0.2% of caffeine, obtainable by the processes of claims 1-12.
14. Green tea polyphenol fractions containing 50-65% epigallocatechin-3-O-gallate, 13-20% epicatechin-3-O-

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gallate, 2-4% epicatechin and 1.5-3% epigallocatechin and no more than 0.2% of caffeine.

5 15. Pharmaceutical compositions containing the polyphenol fractions of claims 13 or 14 in admixture with a suitable carrier or excipient.

16. Pharmaceutical compositions according to claim 15 in the form of tablets, gelatin capsules, sachets, syrup, vials.

10 17. Pharmaceutical compositions according to claims 15 or 16, further containing anthracycline or cis-platinum and derivatives thereof, optionally in a form suitable for the sequential or separate administration.

15 18. Cosmetic compositions containing the polyphenol fractions of claims 13 or 14 in admixture with suitable carriers or excipients.

19. Dietetic compositions containing the polyphenol fractions of claims 13 or 14 in admixture with suitable carriers or excipients.

20 20. The use of the polyphenol fractions of claims 13 or 14 for the preparation of medicaments having differential cytotoxic and antioxidizing activities.

25 21. The use of the polyphenol fractions of claims 13 or 14 for the preparation of medicaments for the treatment of tumoral forms, chronic-degenerative conditions of the cardiovascular system, arthrosic conditions, atherosclerosis.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 96/00973

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K35/78 A61K7/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	DATABASE WPI Section Ch, Week 9537 Derwent Publications Ltd., London, GB; Class B02, AN 95-280843 XP002007502 & JP, A, 07 179 353 (MITSUI NORIN KK) , 18 July 1995 see abstract ---	1-19
Y	DATABASE WPI Section Ch, Week 9147 Derwent Publications Ltd., London, GB; Class B02, AN 91-343154 XP002007503 & JP, A, 03 228 664 (MEIJI SEIKA KAISHA) , 9 October 1991 see abstract --- -/-	1-14, 19

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PCT/EP 96/00973

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Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US,A,4 840 966 (HARA YUKIHIKO ET AL) 20 June 1989	1-17
A	see column 1, line 31 - column 3, line 8 ---	21
Y	US,A,4 673 530 (HARA YUKIHIKO) 16 June 1987	1-19
A	see column 1, line 1 - column 3, line 3 ---	21
Y	US,A,4 248 789 (OKADA FUMIO) 3 February 1981	1-14
	see column 2, line 10 - column 4, line 2 ---	
A	CN,A,1 097 411 (ZHEJIANG MEDICAL UNIV) 18 January 1995 -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PLI/EP 96/00973

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-4840966	20-06-89	JP-C- 1874131 JP-A- 63214183	26-09-94 06-09-88
US-A-4673530	16-06-87	JP-B- 1044234 JP-C- 1561043 JP-A- 59219384	26-09-89 31-05-90 10-12-84
US-A-4248789	03-02-81	JP-C- 1092306 JP-A- 55105677 JP-B- 56038591 CH-A- 642364	16-04-82 13-08-80 08-09-81 13-04-84
CN-A-1097411	18-01-95	NONE	

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